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Effect of Enzymes on the Interaction of Enteroviruses with Living HeLa Cells[1]

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ABSTRACT

ZAJAC, IHOR (Hahnemann Medical College, Philadelphia, Pa.), AND RICHARD L. CROWELL. Effect of enzymes on the interaction of enteroviruses with living HeLa cells. J. Bacteriol. **89**:574–582. 1965.—Eight crude enzyme preparations and two crystalline enzymes were tested for ability to inactivate coxsackie group B and poliovirus receptors on living HeLa cells. Receptor-destroying enzyme, erepsin, lysozyme, collagenase, proteinase, and cobra venom did not alter attachment of coxsackie B3 or poliovirus T1 to cells, whereas elastase prevented attachment of both viruses tested. Treatment of live cells with pancreatin or chymotrypsin rendered cells unable to attach group B coxsackie viruses, whereas cells treated with trypsin failed to attach poliovirus T1. In addition, chymotrypsin was found to release coxsackie B3 and poliovirus T1 from cell surfaces, whereas trypsin was unable to dissociate virus-cell union. These results indicate that cellular receptors for polioviruses differ from those for group B coxsackieviruses. The finding that 1% solutions of enzymes will inactivate differentially the enteroviral receptors of HeLa cells, without altering cell viability, provides a useful approach for study of enterovirus receptors of live host cells.

Extensive studies have been performed with debris prepared from susceptible cells in an attempt to characterize cellular receptors for enteroviruses (Holland and McLaren, 1959; Quersin-Thiry and Nihoul, 1961; Philipson and Bengtsson, 1962; Thorne, 1963). Although these earlier studies have contributed greatly to the knowledge of enteroviral receptors, the disruption of the cells used may have altered the membrane structures to allow the receptors to be nonspecifically inactivated by assorted reagents. Therefore, it was reasoned that a better understanding of the nature of viral receptors and of their function in viable cells could best be elucidated by a study of living cells. In this report, we describe experiments in which enterovirus receptors of live HeLa cells were tested for resistance to inactivation by various enzymes.

[1] Presented in part as a preliminary report before the American Association of Immunologists, Chicago, 14 April 1964. The material in this paper represents in part the thesis submitted by I. Zajac in partial fulfillment of the requirements for the Ph.D. degree.

[2] Research Career Development Award Grantee of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

MATERIALS AND METHODS

HeLa cells. A subline of HeLa cells (designated JJH), adapted to grow in medium containing calf serum, was obtained from J. J. Holland in 1961 and was routinely used in these experiments. Another subline of HeLa cells (MBA) was purchased from Microbiological Associates, Inc., Bethesda, Md., in 1960 and was adapted to medium containing calf serum. Both cell lines were kept, in continuous cultivation in this laboratory, on the flat surface of 32-oz prescription bottles. The growth medium used for cell cultivation contained Eagle's essential and nonessential amino acids and vitamins in Hanks' balanced salt solution (BSS), with 10% calf serum, and 100 units of penicillin and 100 μg of streptomycin per ml. The *p*H was adjusted to 7.2 to 7.4 with sodium bicarbonate. Cells, in monolayers, were serially subcultured at weekly intervals by methods described previously (Crowell and Syverton, 1961).

Determination of cell concentration and cell viability. The concentration of cells in prepared suspensions was determined microscopically by use of a hemocytometer. Trypan blue was routinely added to appropriate dilutions of cells in BSS to give a final concentration of 0.1% to permit determination of the number of viable cells. In some experiments, cell viability was also determined by the efficiency-of-plating (EOP) tech-

nique. For use of this technique, diminishing concentrations of HeLa cells, in 5.0 ml of growth medium containing 20% calf serum and double the usual amount of glutamine, were distributed to replicate screw-cap bottles (30 by 60 mm), and incubated in a stationary, horizontal position at 37 C for 12 days under a gas phase of 5% CO_2-95% air (Puck, Marcus, and Cieciura, 1956). Clones of cells which developed were rinsed free from medium, stained with crystal violet (0.5% in 20% ethanol), and enumerated by use of a Quebec colony counter. The EOP was expressed as the ratio of the number of clones which contained more than 30 cells per clone to the total number of cells inoculated.

Viruses and virus assay. The origin of strains of B1, B3, and B5 coxsackieviruses and poliovirus T1 used in this investigation and the method of assay were described previously (Crowell and Syverton, 1961).

Determination of virus attachment. Cells in monolayers were rinsed twice with phosphate-buffered saline (PBS) and suspended, by use of a rubber scraper and 0.02% ethylenediaminetetraacetic acid (EDTA), in PBS free from magnesium and calcium (Dulbecco and Vogt, 1954*a*) at *p*H 8.0. The cells were sedimented by centrifugation at 225 × *g* in an International centrifuge (model UV) for 5 min, resuspended in BSS, and counted. A sample containing 10^7 cells was transferred to a sterile test tube and centrifuged as before, and the sedimented cells were resuspended in 2 ml of growth medium. A 0.2-ml virus inoculum containing 10^6 plaque-forming units (PFU) was added to the cell suspension, and the mixture was incubated in a water bath at 37 C for 1 hr with intermittent shaking. After 5, 15, 30, and 60 min, a 0.1-ml portion of the cell-virus suspension was added to 10.0 ml of PBS supplemented with 5% calf serum to stop viral attachment, and the diluted cell suspensions were centrifuged at 1,100 × *g* for 10 min to remove the cells. The upper 5.0 ml of the supernatant fluid, containing unattached virus, was stored at −24 C pending virus assay. For use as a standard of initial virus content, a virus control tube containing the virus inoculum and growth medium, in place of the cell suspension, was included in each experiment.

Preparation and assay of cell-associated virus (CAV). Cells grown in monolayers were rinsed two times with PBS and suspended by use of EDTA and a scraper; cells were then sedimented by centrifugation, resuspended in BSS, and counted. A sample of 5×10^7 washed cells was centrifuged at 225 × *g* for 5 min, the sedimented cells were resuspended in 5 ml of virus (1.1×10^8 PFU per ml), and virus-cell interaction was allowed to proceed for 2 min at 37 C. Viral attachment was interrupted by diluting the cell-virus mixture 10-fold with 45.0 ml of cold BSS, and the cells were deposited by centrifugation at 225 × *g* for 5 min at 2 C. The cells were readied for use by washing four times with cold BSS (50 ml each wash) to remove unattached virus. The amount of CAV was determined by the acid *p*H reactivation procedure of Fenwick and Cooper (1962).

Preparation of cell debris. To prepare cell debris, 5×10^7 cells were suspended in 1.0 ml of BSS and subjected to seven cycles of alternate freezing in liquid nitrogen and thawing at 37 C, with vigorous mixing of the cells on a Vortex Junior mixer (Scientific Industries, Inc., Queens Village, N.Y.). Complete disruption of the cells was always obtained, as measured by microscopic inspection of the homogenized suspension. The resultant debris was used at a final concentration equivalent to 5×10^6 cells per milliliter.

Preparation of enzymes and enzyme inhibitors. Fresh enzyme solutions were prepared for each experiment. The enzyme was weighed and dissolved in BSS; the final *p*H of the enzyme solution was adjusted to 7.8 with 0.1 N NaOH, and the preparation kept at 2 C until used. Pancreatin (5× National Formulary), erepsin, proteinase, and lysozyme were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; elastase (bacterial) grade B, and collagenase (bacterial) grade B, were purchased from Calbiochem; α-chymotrypsin (3× crystallized) and trypsin (2× crystallized, lyophilized, salt-free) were purchased from Worthington Biochemical Corp., Freehold, N.J.; cobra venum (Naja naja; lyophilized) was kindly provided by G. Sumyk, Armour Research Institute, Chicago, Ill. A solution of receptor-destroying enzyme (RDE) of *Vibrio cholera* was purchased from Microbiological Associates, Inc., Bethesda, Md.

The specific chymotrypsin inhibitor (Schoellmann and Shaw, 1963), L-1-tosylamido-2-phenethyl chloromethyl ketone (TPCK) was purchased from Cyclo Chemical Corp., Los Angeles, Calif., and was used at a final concentration of 4 mM.

RESULTS

Effect of assorted enzymes on attachment of virus to HeLa cells. Eight crude enzymes were screened separately for ability to inhibit the attachment of coxsackievirus B3 and poliovirus T1 to live HeLa cells. In these experiments, determinations of the effect of enzymes on the ability of cells to attach virus were conducted in the presence of the enzyme. This method of screening enzymes made possible the detection of enzymatic activity, whether it was directed (i) toward viral receptors on cells, (ii) to reversal of virus-cell union, or (iii) to both sites. Presence of residual enzyme in the test fluids, after dilution, did not influence the assays for virus. The results of these experiments (Table 1) showed that RDE, lysozyme, proteinase, erepsin, collagenase, and cobra venom did not alter viral attachment significantly, whereas elastase markedly reduced the ability of cells to bind both of the viruses tested. Pancreatin inhibited the attachment of coxsackievirus B3, but had no apparent effect on

TABLE 1. *Effect of assorted enzymes on virus stability and on viral receptor activity of HeLa cells**

Enzyme	Final enzyme concn	Virus stability to enzyme		Per cent of unattached virus			
				Coxsackie B3		Polio T1	
		Cox. B3	Polio T1	Treated	Normal	Treated	Normal
	%						
Receptor-destroying enzyme	Undiluted	100	ND†	20	10	ND	ND
Lysozyme	0.5	100	ND	2	3	ND	ND
Proteinase	0.5	100	ND	2	4	ND	ND
Erepsin	1.0	100	100	12	9	39	35
Collagenase	0.5	36	100	9	10	20	18
Cobra venom	0.1	100	100	25	10	59	48
Elastase	0.5	100	100	87	11	100	16
Pancreatin	0.5	100	100	37	4	8	10
Chymotrypsin	1.0	100	100	53	2	23	15
Trypsin	1.0	100	100	34	2	25	15

* HeLa cells were washed free from growth medium and suspended; samples containing 10×10^6 cells were distributed to separate tubes. The suspensions were centrifuged, the supernatant fluid was discarded, and the cells were resuspended in 2 ml of the respective enzyme solution. Each tube immediately received 10^6 PFU of virus in 0.2 ml, and the amount of unattached virus was determined at intervals of incubation at 37 C. Similarly, tubes without cells were included to serve as control of virus stability. The data are expressed as per cent of input virus recovered after 60 min of incubation.

† Not done.

that of poliovirus T1. A greater degree of inhibition of the rate of attachment of B3 virus to cells by pancreatin was found in another experiment in which enzyme was incubated with cells for 1 hr at 37 C prior to addition of virus (Fig. 1).

The knowledge that pancreatin contained chymotrypsin and trypsin, and the appearance of a publication by Philipson and Bengtsson (1962), influenced a decision to screen crystalline preparations of trypsin and chymotrypsin for effect on rate of attachment of T1 and B3 viruses to living HeLa cells. Results of these experiments (Fig. 2 and 3, Table 1) revealed that the action of chymotrypsin and trypsin for B3 virus resembled that of pancreatin. In contrast, under the conditions tested, trypsin and chymotrypsin showed no apparent ability to reduce attachment of poliovirus. Poliovirus T1 and B3 coxsackievirus were found to be stable in all of the enzyme solutions tested with the exception of collagenase, which reduced coxsackie B3 infectivity by 64%.

The finding that trypsin did not adversely influence poliovirus attachment in the screening type of experiments was unexpected in view of observations of others in which trypsin treatment of cells reduced the ability of the cells to attach virus (Cooper, 1961) and in which trypsin and chymotrypsin inactivated enteroviral receptors on cellular debris (Holland and McLaren, 1959; Quersin-Thiry and Nihoul, 1961; Philipson and Bengtsson, 1962). In an attempt to relate our findings to these observations and to extend the study of the effect of enzymes on living cells, a systematic investigation of the comparative effects of chymotrypsin and trypsin on the ability of HeLa cells to attach poliovirus T1 and coxsackievirus B3 was conducted.

Influence of concentration of chymotrypsin and trypsin on rate of B3 virus attachment and on cell viability. An experiment was performed to determine the effect of concentration of trypsin and chymotrypsin on the ability of live cells to attach B3 virus. The results (Fig. 4) show that maximal receptor inactivation occurred when the enzymes were used at a final concentration of 1% under the conditions tested. The use of relatively high concentrations of enzymes resulted in an increased recovery of viable cells.

Influence of cell concentration on extent of enzyme action. The finding of incomplete inhibition of attachment of coxsackievirus B3 to HeLa cells by chymotrypsin, even when tested at a concentration of 1%, suggested that the number of cells employed was too great for the enzyme to inactivate all of the viral receptors. In an attempt to increase the inhibitory effect of chymotrypsin on attachment of B3 virus, the enzyme was tested on reduced concentrations of HeLa cells. The results of this experiment (Fig. 5) reveal that attachment of B3 virus to HeLa cells could be completely inhibited by action of 1% chymotrypsin provided that relatively low cell concentrations were employed.

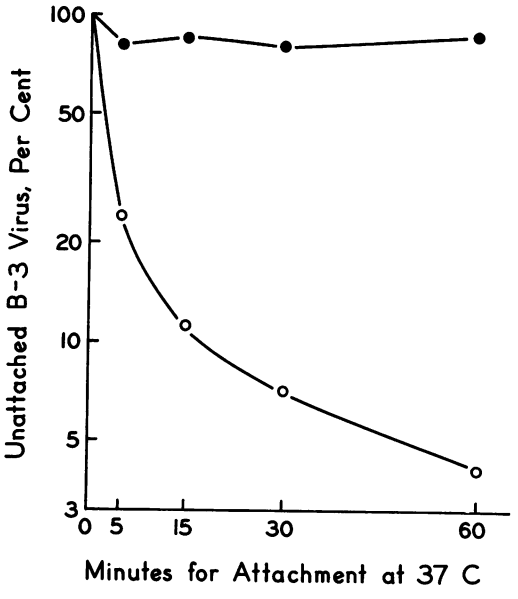

FIG. 1. *Effect of pancreatin on the rate of attachment of coxsackievirus B3 to HeLa cells. This experiment was performed in a manner similar to that described for Table 1, except that the cells were preincubated with pancreatin for 1 hr at 37 C prior to addition of virus. Symbols:* ● = *pancreatin;* ○ = *normal cells.*

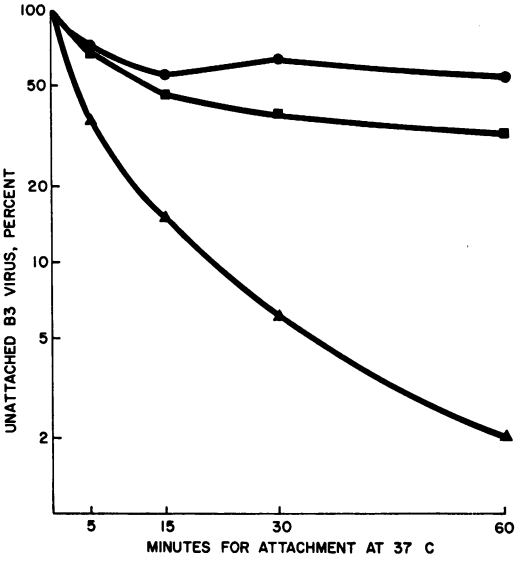

FIG. 2. *Effect of chymotrypsin and trypsin on the rate of attachment of coxsackievirus B3 to HeLa cells. Details of experimental procedure are described under Table 1. Symbols:* ● = *chymotrypsin;* ■ = *trypsin;* ▲ = *normal cells.*

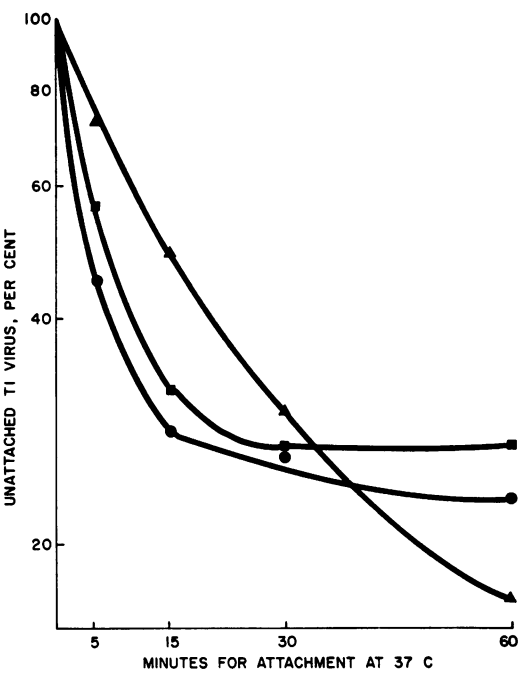

FIG. 3. *Effect of chymotrypsin and trypsin on the rate of attachment of poliovirus T1 to HeLa cells. Details of experimental procedure are described under Table 1. Symbols:* ● = *chymotrypsin;* ■ = *trypsin;* ▲ = *normal cells.*

Relationship between enzyme activity and inhibition of virus attachment. Experiments were performed to establish that the observed inhibition of the HeLa cell-binding capacity for coxsackievirus B3 was due to the enzyme activity of chymotrypsin. Chymotrypsin was rendered inactive by (i) low temperature, (ii) heat, (iii) 20% calf serum, and (iv) TPCK, and was tested for ability to prevent attachment of virus to cells, as described previously. The results shown in Fig. 6 indicate that conditions known to inhibit chymotrypsin activity removed completely, or reduced, the ability of chymotrypsin to alter the rate of attachment of B3 virus to cells.

Effect of enzyme treatment on ability of cells to form clones. As shown previously by use of trypan blue, treatment of HeLa cells with trypsin or chymotrypsin employed at a concentration of 1% had a stabilizing effect on cell viability. To confirm this observation, cell viability of treated cells was determined by testing the ability of cells to form clones. Results of these assays (Table 2) show that chymotrypsin did not decrease the EOP of the two sublines of HeLa cells tested. It is significant that the JJH subline, used routinely in this study, was found to grow very well in mass

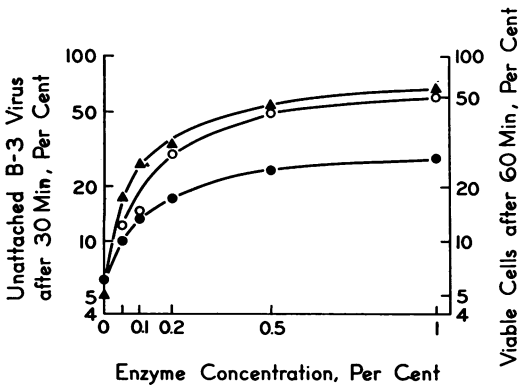

FIG. 4. *Effect of concentration of chymotrypsin and trypsin on the rate of attachment of coxsackievirus B3 to HeLa cells and on cell viability. Washed HeLa cells were suspended in 2-ml volumes of the respective enzyme solution to give a final cell concentration of 5 × 10⁶ per ml. Coxsackievirus B3 was immediately added to each cell suspension in a 0.2-ml volume (10⁶ PFU), and the amount of unattached virus remaining after 30 min of incubation at 37 C was determined. The concentration of viable cells remaining after 60 min of incubation was determined by aid of trypan blue. Symbols: ▲ = chymotrypsin; ● = trypsin; ○ = viable cells as found for each enzyme.*

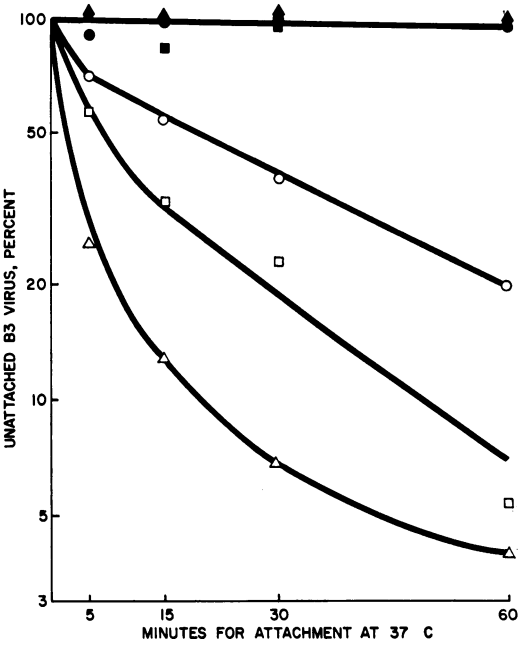

FIG. 5. *Ability of chymotrypsin to inhibit completely the attachment of B3 virus to HeLa cells. This experiment was performed in a manner similar to that described under Table 1. Solid symbols represent cells treated with chymotrypsin; open symbols, normal cells; circles, 5 × 10⁵ cells per ml; squares, 10⁶ cells per ml; triangles, 3 × 10⁶ cells per ml.*

cultures, but gave relatively low EOP recoveries in repeated assays of dilute cell suspensions.

Nature of enzyme inhibition and differential inactivation of viral receptors. In previous experiments, the method of testing enzymes for inhibition of virus attachment to cells did not determine whether enzyme directly inactivated cell receptors, or released virus by reversal of virus-cell union, or both, since the assays were performed in the presence of enzyme.

Experiments were conducted in which HeLa cells were preincubated for 1 hr at 37 C with enzyme and in which the enzyme was removed prior to the determination of the kinetics of virus attachment. The results (Fig. 7) reveal that chymotrypsin completely inhibited the ability of the cells to attach B3 virus, whereas attachment of poliovirus T1 was only partially inhibited. Conversely, pretreatment of cells with trypsin caused total inhibition of attachment of poliovirus T1, whereas the rate of attachment of B3 virus was partially reduced (Fig. 8). Similar results were obtained from repeated experiments. Additional group B coxsackieviruses, B1 and B5, were tested for ability to attach to chymotrypsin-treated cells in experiments of similar design; the findings were the same as for B3 virus. In another experiment performed to measure the rate of B3 receptor inactivation of HeLa cells by chymotrypsin, 15 min of enzyme treatment was found adequate to render the cells unable to attach virus. These results indicate that enzymes inactivate viral receptors on the surface of live cells and that poliovirus receptors can be distinguished from those for group B coxsackieviruses on the basis of differential enzyme inactivation.

The ability of chymotrypsin and trypsin to release B3 and T1 viruses, respectively, from the surface of live HeLa cells was tested. Washed cells with attached virus were suspended in 1% enzyme solutions at 37 C, and free virus was measured at intervals of incubation. The amount of each virus present initially at the cell surface was ascertained by treating samples of the virus-cell suspensions in glycine buffer at *p*H 2.5, according to the acid pH reactivation method of Fenwick and Cooper (1962). The amount of each virus obtained at *p*H 2.5 at zero time was considered, for reference, as the total amount of virus which would be available for release by enzyme. The results (Fig. 9) show that chymotrypsin released both B3 and T1 viruses from the

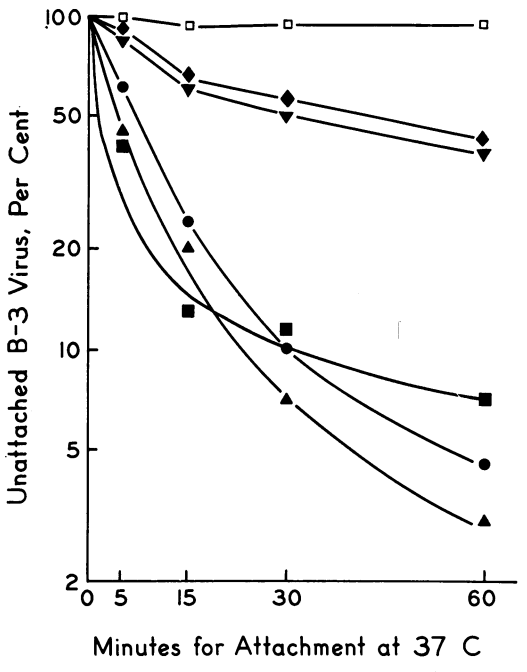

FIG. 6. *Relationship between chymotrypsin activity and inhibition of attachment of coxsackievirus B3. To test the effect of low temperature on chymotrypsin activity, cells were suspended in 1% enzyme at a concentration of 3 × 10⁶ per ml and incubated for 1 hr in an ice bath at 2 C. The cells were washed, resuspended in growth medium with 20% calf serum (GM-CaS20) and tested for ability to attach virus. Chymotrypsin, which had been heated in a boiling-water bath for 5 min, or dissolved in GM-CaS20, or preincubated with TPCK for 2 hr at pH 7.2, 24 C, was tested, separately, as described previously. Cells suspended in GM-CaS20 served for control of normal virus attachment. Symbols:* □ = *chymotrypsin;* ◆ = *enzyme, GM-CaS20;* ▼ = *enzyme, 2 C;* ● = *enzyme, heated;* ■ = *enzyme, TPCK;* ▲ = *normal.*

surface of cells. The finding of amounts of B3 virus greater than 100% after chymotrypsin treatment suggests that the *p*H 2.5 reactivation procedure did not measure all of the B3 virus which was cell-associated at zero time. Trypsin, on the other hand, did not reverse virus-cell union for either virus tested. This latter finding is in agreement with earlier observations of Holland (1962) and Philipson and Bengtsson (1962), who reported that trypsin failed to release poliovirus T1 attached to cell debris.

Results of these experiments indicate that chymotrypsin inactivates B3 coxsackievirus receptors on live HeLa cells and, in addition, is able to release B3 and T1 viruses bound to cell

TABLE 2. *Effect of 1% chymotrypsin on ability of HeLa cells to form clones**

Duration of incubation	Efficiency of plating			
	HeLa cell-MBA		HeLa cell-JJH	
	Normal	Treated	Normal	Treated
min	%	%	%	%
1	109	—	27	—
15	133	122	14	21
30	139	144	15	33
60	146	140	15	34

* HeLa cells of the JJH or MBA sublines were suspended at a concentration of 2.5 × 10⁶ cells per milliliter in 1% chymotrypsin, and the efficiency of plating of the cells was determined, in triplicate, at intervals of incubation at 37 C. Normal cells suspended in growth medium, containing 20% calf serum and double the usual amount of glutamine, without enzyme served as control.

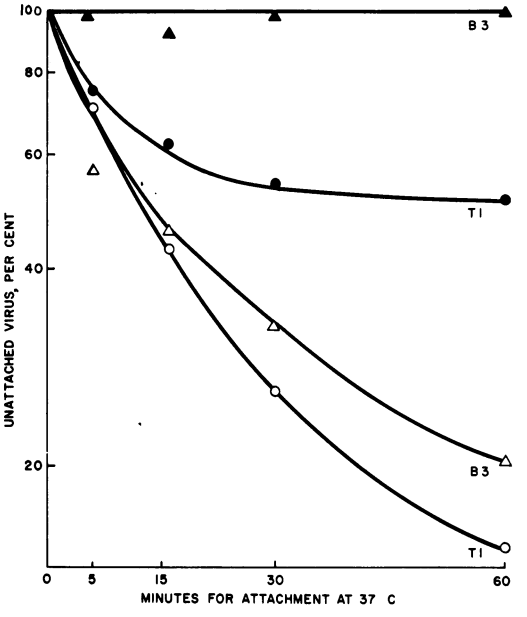

FIG. 7. *Comparative effect of pretreatment of HeLa cells with chymotrypsin on the rate of attachment of coxsackievirus B3 and poliovirus T1. Cells were suspended in 1% chymotrypsin at a concentration of 3 × 10⁶ per ml, incubated at 37 C for 1 hr, diluted eightfold in growth medium with 20% calf serum (GM-CaS20), and the cells were deposited by centrifugation. The cells were washed twice in 50-ml volumes of GM-CaS20 and tested for ability to attach virus at a final concentration of 3 × 10⁶ per ml. Solid symbols represent cells treated with chymotrypsin; open symbols, normal cells.*

surfaces. On the other hand, trypsin inactivates the ability of live HeLa cells to attach poliovirus T1 and has no ability to release either virus tested from the cell surface. The specific mechanism by which enzymes inactivate the ability of cells to attach virus remains to be determined.

DISCUSSION

In an attempt to study enterovirus receptors on living host cells in a manner similar to that applied to myxovirus receptors of whole erythrocytes (Burnet and Stone, 1947), crude enzyme preparations, which contained an assortment of enzymes, were used initially with the expectation that a preparation would be found which would inactivate enteroviral receptors. This approach was rewarded by the finding that pancreatin and elastase contain enzymes which inhibit the attachment of poliovirus and coxsackieviruses to HeLa cells. Results of subsequent experiments in which crystalline enzyme preparations were used showed that treatment of cells with chymotrypsin rendered HeLa cells unable to attach B3 virus, whereas the attachment of poliovirus T1 was affected minimally. Trypsin, on the other hand, was found to inactivate specifically the cellular receptors for poliovirus T1. It is significant that this differential inactivation of enterovirus receptors was not found when HeLa cell debris was used as a source of receptor substrate (Holland and McLaren, 1959; Philipson and Bengtsson, 1962; Zajac and Crowell, *unpublished data*).

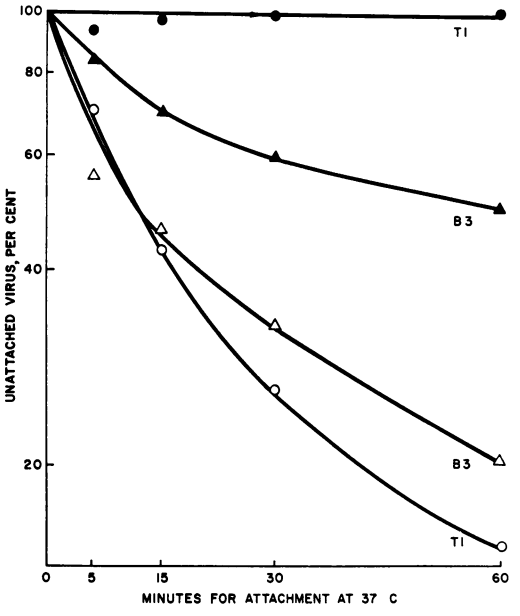

FIG. 8. *Comparative effect of pretreatment of HeLa cells with trypsin on the rate of attachment of coxsackievirus B3 and poliovirus T1. Details of experimental procedure are described under Fig. 7. Solid symbols represent cells treated with trypsin; open symbols, normal cells.*

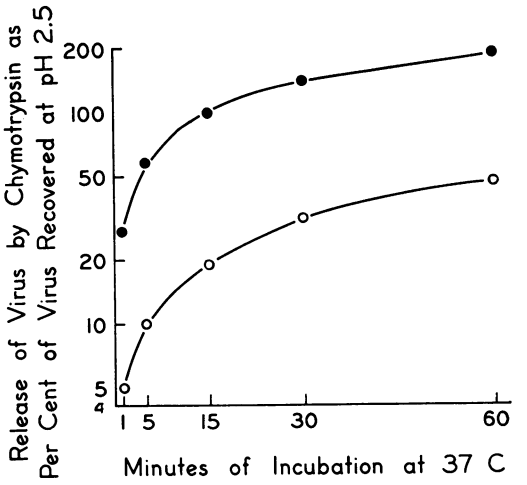

FIG. 9. *Ability of chymotrypsin to release coxsackievirus B3 and poliovirus T1 from the surface of live HeLa cells. Washed cells with attached virus (see Materials and Methods) were suspended in 1% enzyme at a concentration of 3 × 10^6 cells per ml and incubated at 37 C. Samples were removed at intervals, diluted 100-fold in PBS with 5% calf serum and centrifuged; the supernatant fluid was assayed for unattached virus. The amount of each virus present at the cell surface at zero time was determined by diluting the virus-cell suspensions 100-fold into 0.05 M glycine buffer (pH 2.5) for 1 min at 20 C, and assaying for unattached virus after neutralization of pH and removal of cells by centrifugation. The amount of virus released by chymotrypsin is expressed as per cent of the amount of virus recovered at zero time by glycine buffer. Symbols: ● = coxsackievirus B3; ○ = poliovirus T1.*

The interpretation that specific receptors on living cells are inactivated by enzymes, without reducing cell viability, is supported further by results of additional experiments (Zajac and Crowell, 1964) which have revealed that enzyme-treated cells will regenerate specific receptor activity when the cells are returned to a normal environment. This regenerative capacity of cells, together with the finding that relatively large amounts of enzyme are required for complete inactivation of viral receptor activity, would account for the fact that routine use of low amounts of trypsin for dispersion of cells for tissue-culture systems has been infrequently observed (Dulbecco and Vogt, 1954*b*; Cooper, 1961) to be detrimental for virus studies.

It is of interest that pretreatment of cells with trypsin was found to inactivate the cellular

receptor activity for poliovirus, whereas trypsin apparently accelerated the rate of attachment of poliovirus to cells when tested in the screening type of experiment (Fig. 3). Further experiments are necessary to resolve this paradox, although it is tempting to speculate that, in the latter type of experiment, trypsin may have exposed additional receptors during the initial phase of its action on the cell surface.

Suspensions of HeLa cells prepared in 1% solutions of proteolytic enzymes initially became highly viscous (Easty, Easty, and Ambrose, 1960) when incubated at 37 C. The cells were observed to clump, and the *p*H of the fluid phase decreased. As incubation continued, the viscosity of the medium decreased, and the cells became redispersed. Weiss (1958) demonstrated that trypsinization of sarcoma 37 cells resulted in a 20% loss of the dry mass of the cells; this loss was attributed to detachment of a slime coat from the cells. Trypsinization of erythrocytes resulted in a 20% reduction of net negative charge (Ponder, 1951), which has been considered to be due to the release of a sialomucopeptide from the cell surface (Cook, Heard, and Seaman, 1960). In addition to altering the surface of erythrocytes (Springer, 1963), proteolytic enzymes act on the surface of living HeLa cells, with liberation of proteinaceous substances including myxovirus hemadsorption-reactive substance (Marcus, 1962) and species-specific antigenic material (Weiss, 1963). Whether cellular receptors for enteroviruses are associated with these substances remains to be determined.

To account for the observed 50% reduction in the rate of attachment of B3 virus to cells treated with trypsin and for the corresponding reduction in attachment of poliovirus to cells treated with chymotrypsin, it is tempting to postulate that these results were due to a nonspecific decrease in effective cell surface area. It is also possible that the cross-reactivity of the enzymes was due to (i) inactivation of overlapping viral receptor areas on the cell surface or (ii) inactivation of a common substrate which existed in different proportions within the respective receptor areas. Nevertheless, it was concluded that poliovirus receptors on living cells can be differentiated from those for group B coxsackieviruses on the basis of differential susceptibility to enzyme action. This conclusion is consistent with previous findings, which revealed that major differences exist between receptors for group B coxsackieviruses and for polioviruses (Crowell and Syverton, 1961; Quersin-Thiry and Nihoul, 1961; Philipson and Bengtsson, 1962; Crowell, 1963*a*, *b*).

ACKNOWLEDGMENT

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